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THE CONTROLLING INFLUENCE OF SEED RATE PROPORTIONALITY OF COMPANION CROPS ON THE OUTTURN WHEN GROWN MIXED

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(Received on 26th August 1957)

INTRODUCTORY

Contrary to the preponderating agricultural practice of monoculture of crops in the temperate regions, mixed cropping, namely, growing of two or more plant species in deliberate association, is traditional to India and has been widely practiced ever since the time of *Rigveda* (2·000 to 1,400 B.C.). Indeed mixed cropping is a tabloid form of rotation where the crops grown in association alternate in space, though not in time. In suiting the varying soil and climatic conditions of this vast sub-continent, presumably no single crop husbandry practice has played such a vital role in rehabilitating soil conditions and increasing crop yield as mixed cropping.

The control exercised by the severity of climate, and, in particular, of soil temperature, soil moisture, day length, and night temperatnre during the period of growth have influenced considerably the choice of crops and cropping practices. The smallness of holdings, generally poor soils of low organic matter content, uncertain weather conditions, inadequate and often failing water supply, the pressing economic needs of the cultivator to produce his requirements of sugar, starch, oil, protein and fibre, to suit usually vegetarian mode of life, have favoured the practice of mixed cropping.

In an endeavour to increase or to conserve soil fertility, as fast as it deteriorates, under the stress of tropical and sub-tropical conditions and yet to obtain as large a yield, as is economic, or possible, the inexpensive practice of mixed cropping has contributed materially in soil building and crop production under semi-arid conditions. Introduction of legum s in association with non-legumes, with varying root lengths of plant species drawing nutrients from different depths, has found marked favour in the evolution and choice of appropriate crop mixtures.

Notwithstanding the earliest recognition of the prominent part played by mixed cropping and the unique position which it occupies in our agricultural economy, covering almost one-sixth of the cropped area under differing conditions of soil and climate, there is great paucity of scientific information on the subject. A precise knowledge of the method and technique for maintaining optimum growth conditions and the determination of the most favourable association of plant species and seed rate proportionality in the crop mixture is the first step to any planned utilization of land for nitrogen economy and larger produce.

In view of the conflicting results on the utility of mixed cropping and biological fixation of nitrogen by legumes, obtained in different parts of the world, and in particular, owing to great lack of precise information under the Indian conditions. the need was felt to advance experimental evidence to the elucidating of this traditional practice and providing the scientific principles governing it.

In the analysis of the several factors governing the growth performance and yield of a crop, in field experimentation, on a limited land of known fertility and under a given set of cultural conditions, perhaps no other single factor exercises such controlling influence as plant population within the unit space competing for nutrients and water below ground and light above it. In arable farming, the plant population is determined primarily by the dominating control of the seed rate. The seed rate proportionality (SRP) between wheat (W) and gram (G), or *gochana,* the most widely practised crop mixture in the country, has stood in variance and wide apart from one state to another in the country. Thus, seed rate pro portionality (by weight) practised between wheat and gram* alone are W: G: :1:1, 2:1, 10:1 in Hyderabad ; 1:1, 1:1.5, 1:2 in Bengal ; 1:1, 2:1, 3:1, 4:1 in the Punjab ; 1:1, 2:1, in Assam ; 4:3 in Bhopal, 1:4 in Bombay and 1:1 to 6:1 in Uttar Pradesh (*c.f.* Iyer, 1949). With such wide fluctuations in the seed rate proportionality of the mixture it is not possible to know precisely as to which ratio would be optimum for highest return and fertility value of the soil.

Prior to the analysis of the several conditioning variables on growth characteristics and yield of the crops sown mixed, it became imperative to first extricate the effect of varying seed rate ratios on the growth and yield of the companion crops singly and collectively. The interaction between plants growing together is liable to be controlled by the companion crops as also the seed rate proportionality between these. Thus, before giving a verdict on the eligibility of the practice of mixed cropping with regard to a set of companionships and seed rates under a known set of soil and climatic conditions, it becomes atonce necessary to know the relative growth response of the companion crops grown together in terms of outturn of seed and forage.

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* Throughout the paper seed rate proportionality has been stated in the order Wheat : Gram or (W:G).

PROCEDURE

A simple randomised replicated field experiment was laid out on the unmanured sandy loam soil, typical of the *Gangetic alluvium,* of moderate fertility. The field was divided into nine plots, each of 1/40 acre, corresponding to the number of seed rate combinations tried. No special cultural or inter-cultural operations, other than the customary, were done to ensure maximum applicability of the results on the cultivators' holdings.

Determination of the optimum seed rate proportionality as between wheat (W) and Gram (G) was secured with a number of combinations of the seed rates of the two companion crops as 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5 by weight. Each unit in the ratio was equivalent to 8 lb of seed/acre, *e.g.*, W:G: 5:1 represented 40 lb of wheat+8 lb of gram, sown (mixed, on an acre basis, likewise, W:G: 1:5 corresponded to acre seed rate of 8 lb of wheat+40 lb of gram sown together.

RESULTS AND DISCUSSION

Analysis of data revealed that the yield of wheat grain was maximum (11·63 md*/acre) and significantly so with the mixture ratio of W:G: 4:1 (Table 1), the grain yield due to the remaining mixture rates in order being 3:1 > 5:1 > 2:1 > 1:1 > 1:1 > 1:2 > 1:3 > 1:4 > 1:5.

TABLE 1.

Influence of seed rate proportionality between wheat and grain

(Yiled, md/acre)

S.R.P. (W:G)	WHEAT GRAIN					WHEAT STRAW				
	Replicates					Replicates				
	I	II	III	IV	Mean	I	II	III	IV	Mean
5:1	10·46	10·94	9·08	11·32	10·45	21·22	18·92	19·74	20·48	20·14
4:1	11·36	11·58	12·72	10·88	11·63	20·74	21·50	22·52	21·28	21·51
3:1	10·48	10·36	11·24	10·44	10·63	20·62	21·22	22·21	20·06	21·00
2:1	10·36	10·08	10·12	10·02	10·14	19·56	17·48	19·24	17·15	18·36
1:1	8·54	7·92	9·24	8·76	8·62	16·88	16·06	18·10	19·06	17·48
1:2	6·92	8·10	6·54	6·02	6·89	12·72	12·76	12·52	12·15	12·54
1:3	4·38	4·64	4·28	4·34	4·41	8·00	8·24	9·32	8·30	8·47
1:4	4·24	4·28	4·30	4·04	4·22	6·48	6·36	6·32	6·21	6·34
1:5	3·20	3·86	3·74	4·02	3·76	5·24	4·56	4·95	6·12	5·22
SE=0·3009	CD=0·8782					SE=0·4176	CD=1·2188			

* 1 md = 82 lb. (approximately).

Increase in the seed rate proportion of wheat in the mixture W:G from 2:1 to 3:1 or 5:1 resulted in no significant difference in yield. Minimum wheat grain (3·76 md/acre) was produced with the ratio 1:5 a proportionality companionship that registered significantly maximum yield of 14·51 md/acre of gram seed (Table 2). Maximum wheat straw (21·51 md/acre) was produced with the seed rate ratio of 4:1 followed by that under 3:1, with no significant difference between the two (Table 1) though the yield under 4:1 seed rate proportionality was significantly greater than the other seed rate combinations. For gram straw also 4:1 ratio proved to be significantly superior to the rest with an yield of 19·32 md/ acre (Table 2).

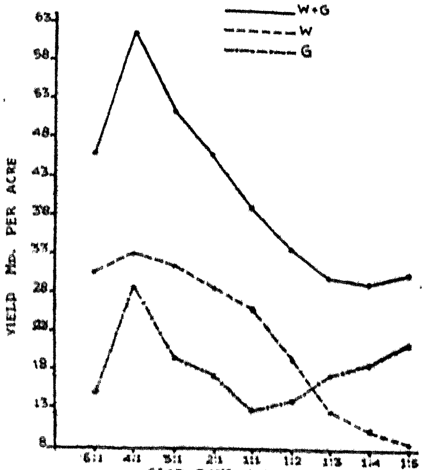

FIG.1. THE INTERACTION OF CROPS IN DIFFERENT SEED RATE RATIOS ON OUT TURN

For achieving maximum combined yield of grain and straw of the two companion crops singly and also collectively, the seed rate proportionality W:G: 4:1 proved best yielding 33·14 md of wheat and 28·53 md of gram, totalling 61·67 md/acre. (Fig. 1). Rise in the proportion of gram seed in the mixture resulted in the gradual lowering of the combined yield, reaching a minimum of 12·95 md/acre at SRP 1:1. With further increase in the seed rate proportionality of the gram component (1:5), there followed a progressive increase in its combined yield, attaining a maximum of 21·75 md/acre (Table 2), evidently due to marked increase in the seed outturn of gram.

TABLE 2

Influence of seed rate proportionality between wheat and gram
(Yield, md/acre)

SRP (W:G)	GRAM SEED					GRAM STRAW				
	Replicates					Replicates				
	I	II	III	IV	Mean	I	II	III	IV	Mean
5:1	4·65	5·75	5·80	4·62	4·70	10·32	12·76	8·45	10·25	10·44
4:1	9·24	8·20	10·23	9·18	9·21	19·39	17·20	21·46	19·25	19·32
3:1	8·63	7·59	7·54	6·78	7·63	13·44	11·83	11·75	10·58	11·90
2:1	8·48	7·23	7·04	6·39	7·28	11·72	10·00	9·86	8·76	10·09
1:1	8·71	7·56	8·48	9·62	8·59	4·42	3·84	4·31	4·86	4·36
1:2	9·56	8·38	9·27	10·78	9·49	4·79	4·20	4·69	5·39	4·77
1:3	11·74	12·62	10·89	10·85	11·77	5·82	6·25	5·00	5·45	5·63
1:4	12·64	12·62	11·79	13·38	12·61	6·32	6·30	6·34	6·72	6·42
1:5	14·53	15·47	14·65	13·40	14·51	7·25	7·72	7·32	6·66	7·24

SE = 0·4198 CD = 1·2252 SE = 0·5308 CD = 1·5491

Contrary to the yield performance of gram, less marked differences were noticeable in the total produce of wheat with change in seed rate ratios. With increase in the seed rate proportionality of the legume beyond 1: 1, the cereal yield declined steadily ; this is in contrast to what was noticed for the legume associate with increase in the cereal seed ratio.

Increase in the seed rate proportionality of the legume associate beyond 1 : 1 and 1 : 4 increased its seed and straw respectively to the detriment of that of the cereal companion (Table 2) through the reverse did not hold good. These findings are in general agreement with those of Mann and Barnes (1953) to the extent that progressive increase in the ratio of legume partner diminished the yield of the other in proportion to the increase affected. The findings of Dungan *et al* (1942) to the effect that growth of legume (red clover) was poorer as the sowing rate of cereal (Gopher oats) was increased could not be substantiated in the present investigation in that with increase in the seed rate of the cereal better growth and higher yield of the legume was attained. Grain and straw yield of wheat, straw yield of gram and also the total combined produce the two associate crops was maximum at W; G: :4: 1 which thus proved to be the optimum (Tables 1, 2).

With rise in the proportion of the legume in the mixture, the yield of the legume seed was increased while that of its straw diminished. The reduction in yield of the legume seed with increase of the cereal companion in the seed mixture was amply compensated by the produce of straw (Table 2) so that the optimum for maxmum outturn was shifted to be achieved with more cereal component. The growth and yield of either associates was affected adversely and the nitrogen excretion declined, were the mixture components not sown in favourable ratio (Tables 1,2).

Contrary and conflicting evidences have been put forth on the question as to whether legume seeding was favoured by sowing together cereal at reduced rates. Thatcher and coworkers (1937) reported that in dry seasons a thin sowing rate of oats my result in better stand of legumes while in other seasons no significant difference in legume stand was observed. Mann and Barnes (1953) found that the presence of the legume reduced the associate crop by an amount diminishing as the density of the latter was increased. Dungan *et al* (1942) reported that increase in the cereal component reduced the straw and growth of the clover. Pendleton and Dungan (1953), on the other hand, did not observe any significant difference in the growth of the legume attributable to difference in row spacing of cereals.

Lyon, Modhok, Calma and Tiangsing, and Osserwande (cf. Iyer, 1949), among others reported negative evidence of benefit from legume-non-legume association while Gurski, Tacke, Lyon and Bizzell (*cf.* Nicol, 1936) have gathered evidences to the contrary. In almost 80 percent cases Lipmann reported no beneficial action of the legume on the non-legume whereas he gave proof of the ability of the non-legume to secure an adequate supply of nitrogen when in association with a legume in nitrogen less sand culture. In their experiments on mixed cropping between species of grasses and legumes, Aberg, Johnson and Wilsie (1943) found that responses of crops held in various asoociation in the green house and in the field were in many cases reversed ; this indicated the importance of evironmental practices in crop behaviour under competition.

Iyer (1949) cited examples from different parts of the country, of profitable, unprofitable and inconclusive results of the effect of WG association against W and G grown pure on the basis of "financial advantage" alone. Virtanen and

his associates investigated the growth of peas and oats in the ratio 1, 2, 2.75, 4 and 5 oat plants per pea plant and reported that as the ratio of non-legume to the legume approached or excreted 2: 1 the growth of both species suffered and in the above case the setback to pea was most evident when the cereal was numerically preponderant (*cf.* Iyer, 1949). The diversity in these results may be due to defective seed rate proportionality unsuited to locality conditions.

The present investigations throw considerable light on the subject and suggest, as stressed elsewhere by the author earlier (Singh, 1954), that in any attempt to grow a deliberate mixture of crop associations for profit two important aspects, *viz.*, the proper choice of companion crops and the maintenance of optimum seed rate proportionality become pre-requisite.

Larger returns with 4: 1 seed rate raito may partly be due to the improved activity of the legume root nodules to the increased absorption of nitrogen by the competing roots of wheat, thereby inducing more excretion (Singh, 1951). Lowering of the seed rate of the legume and the consequent raising of the associate cereal, increased straw yields of both the components indicating pointedly, though indirectly, that under the condition presumably more nitorgen was made available to the growing plants for vegetaive growth ; this is obviously due to greater fixation of nitrogen, in mixed cultures by the legume-*rhizobia* (Singh, 1951). It has been demonstrated elsewhere by the author, working on the 'Performance and Fate of Fertilizers in Agronomic Practice' (1942), that the growing wheat plant was capable of enriching the soil in its nitrogen status through fixation of free nitrogen. In another series of experiments it has been shown by the author that the wheat plant iduced fixation of atmospheric nitrogen to a greater extent when in association with gram (Singh, 1954).

With the introduction of an additional crop, competition sets in with one another but in mixed cropping, where mixtures are introduced deliberately, each crop is expected to make its own contribution by way of produce through mutual adjustment and also by making differing demand on the soil for nutrients and water from varying depths. Where the choice of companion crops is faulty, the mixture is a failure to the reduction of both yield and soil fertility. The result may be a net loss, were the nutrients more in demand by the competing crops than the single culture, unless one of the crops was a legume capable of fixing atmospheric nitrogen in the soil to the advantage of the companion crops. The proper adjustment of soil fertility could thus be achieved through the control of seed rate proportionality in mixed cropping.

Competition for light, as stressed be Stahler (1948) and Pritchett and Nelson (1950), was noticed during the course of extended invastigations on mixed cropping not to be serious in these regions as the gram plant could carry on its photosynthetic activity under relative shading (Singh, 1951); again, the assimilating leaf surface of wheat was increased significantly when grown in association with gram as against its single culture (Singh, 1951) rendering greater carbon assimilation and better dry matter production.

While nitrogen excretion is important, the fact can not be overlooked that plants excrete through the roots certain other compounds which may stimulate or retard the growth of micro-organisms in the rhizosphere; such stimulation might be of immense value if it encouraged the growth of bacteria such as *azotobactor* or organisms whose activities liberated materials beneficial to the plant, either directly by furnishing growth stimulants or indirectly through the mineralization of soil. It may become disadvantageous, were mixed cropping undertaken in a proportion so as

to introduce hard competition for limited nutrients in the soil. In effect, thus, SRP and excretion of toxic compounds are equally operative factors in root interactions. But so long as the competition is not very great, *i. e.*, the proportion of legumes to non-legumes in seed mixture adverse for the legume, gram-wheat mixtures are likely to enrich the soil (Singh, 1951) as also to give improved yields in quantity and in quality.

The growth of the legume was best in association with the non-legumes particularly in respect of grain yield and grain/straw ratio of the produce. The findings unmistakably point out that the grain/straw ratio was a function of the seed rate proportionality, possibly through the controlling influence that it exercised over the carbohydrate content of the plant; with increase in seed rate of gram, the grain/straw ratio decreased. These findings indicate that the non-legumes grow better in association with the legume while the legume stands to lose little owing to the association of a companion non-legume.

The factors involved in the competition for root space below ground were observed in another series of experiments on mixed cropping, not to be of a serious nature in the W:G partnership though it proved to be of significant importnce in the wheat-mustard and mustard-gram mixtures (Singh, 1951).

The determination of the seed rate proportionality, thus, becomes pre-requisite to any understanding of the growth behaviour of competing crops in particular reference to the outturn and also the gain in soil ferility through symbiotic activity of the *Rhizobia* leading to the fixation of atmospheric nitrogen.

Agricultural crops have, thus, not to be sown in association solely on their ability to withstand the competition from other stands growing together but also from the point of view of their relative seed rates that would operate favourably upon the associates to the advantage of the yield of the crops and also soil fertility.

SUMMARY

A field study was undertaken to evaluate the influence of seed rate proportionality in legume-cereal combination and determine the optimum ratio. For highest returns companion crops became a function of their seed rates which determined the yield of each of these and also the overall total produce; it had important bearing upon the grain/straw ratio. With the increase in the legume—cereal ratio, the seed yield of legume component consistently increased while straw yield declined, reverse was true when the cereal component was increased. Optimum ratio under the conditions of the experiment was W:G :: 4 : 1. The loss in seed yield of gram due to the increase of the cereal component in the mixture was amply compensated by the increase in straw yield obviously due to the better availability of nitrogen occasioned by the mixture ratio. The need of favourable partership as also the seed rate proportionality between associate crops is emphasized.

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THE INFLUENCE OF VITAMINS ON THE GROWTH AND SPORULATION OF *PHYLLOSTICTA CYCANDINA* (PASS) AND *P. ARTOCARPINA* (SYD ET BUTL)

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(Received on 15th January 1957)

INTRODUCTION

Various investigators including Robbins and Kavanagh (1942), Fries (1948), Hawker (1939), Mathur et al (1950), Beckman et al (1953), Sadasivan and Subramanian (1954), Grewal (1954) and Agarwala (1955), have shown the importance of vitamins in the nutrition of fungi. Some fungi are able to synthesize, their vitamin requirements from pure chemical substances, while others are incapable of doing so and they need external supply of vitamins for normal growth. Robbins and Ma (1944) reported that some fungi suffer from complete deficiency i.e., they fail to grow in absence of vitamins, while others suffer from partial deficiency only i.e. they can grow slowly and poorly in absence of vitamins. Schopfer (1934) noted that *Phycomyces blakesleeanus* could not grow on pure synthetic media, without addition of leaf extract or thiamin. Marloth (1931) found that growth of *Penicillium digitatum* increased greatly after addition of citrus juice to the culture medium, Hawker (1939) reported that *Melanospora destruens* needed biotin for good growth and thiamin for satisfactory development of perithecia. Grewal (1954) and Agarwala (1955) also observed that concentration of vitamins greatly influenced not only the growth but sporulation also. As both growth and sporulation of a fungus may depend on presence of vitamins, it was considered desirable to study the effect of various concentrations of some vitamins on the growth and sporulation of two species of *Phyllosticta* causing leaf spot diseases.

MATERIALS AND METHODS

The two species of *Phyllosticta* viz., *P. cycadina* and *P. artocarpina* were isolated from diseased leaves of *Cycas revoluta* and *Artocarpus heterophyllus* respectively. Single spore cultures of these organisms were prepared by the dilution method and bacteria were removed by the method described by Brown (1924). Asthana and Hawker's medium A (glucose 5·0 gms, KNO_3 3·5 gms, KH_2PO_4 1·75 gms, $MgSO_4 \cdot 7H_2O$ 0·75 gm, distilled water 1 litre) was used as the basal medium. The method used by Mathur et al (1950) was followed for removing the traces of growth substances from the basal medium. Various vitamins were added singly in the vitamin free medium before autoclaving, except biotin which was aseptically added after autoclaving. Six vitamins viz., B_1, B_2, B_6, C, H and nicotinic acid were added at the rate of 10, 50, 100, 200 and 500/μg per litre. For the study of dry weight, fixed quantity of medium viz. (25 ml) was taken in each 150 c.c pyrex flask. Inoculations were made by adding 0·25 c.c of heavy spore suspension (containing about 200 spores). Cultures were incubated for 15 days at 25°C and the entire fungal mat was then harvested. The dry weight was determined by the method described by Tandon and Bilgrami (1954). Four replicates were used for each treatment.

Standard error and critical differences in growth at various concentrations of vitamins were calculated by the following formulae:

$$\text{Standard error (S.E)} = \sqrt{\frac{\text{mean square of the error}}{\text{number of replicates.}}}$$

and critical difference (C.D.) $= \text{S.E.} \times t \times \sqrt{2}$

The general mean of the table $\pm$ C.D. at 1% level has been graded as moderate. Greater growth than the moderate has been graded as good and lesser one as poor. S.E. and C.D. at 1% level have been recorded.

The degree of sporulation has been indicated on the basis of the number of spores present under the low power field of the microscope.

Number of Spores	Sporulation
Nil	Absent
1-10	Poor
11-20	Fair
21-30	Good
Above 30	Excellent

OBSERVATIONS

The effect of five different concentrations of six vitamins viz. thiamin (B_1), riboflavin (B_2), pyridoxine (B_6), ascorbic acid (C), biotin (H) and nicotinic acid on the growth and sporulation of *Phyllosticta cycadina* and *P. artocarpina* has been summarized in tables 1 and 2 respectively. Separate controls were taken for each series.

TABLE No. 1

Showing average growth in mgs. and sporulation of *Phyllosticta cycadina* at different concentrations of six vitamins. The nature of sporulation is indicated in breackets.

Vitamins	Amount of vitamins in μg per litre					Average
	10	50	100	200	500	
Thiamin B_1	69·0 (Good)	78·0 (Excellent ...	91·0 ...	111·0 ...	123·0 ...)	94·4
Riboflavin B_2	54·0 (Fair)	62·0 (Good ...	71·0 ...	83·0 ...	87·0 ...)	73·4
Pyridoxine B_6	63·0 (Fair)	75·0 (Good ...	89·0 ...)	74·0	60·0 (Excellent)	72·2
Ascorbic acid C	49·0 (Fair ...	44·0 ...	36·0 ...)	33·0 (Poor ...	29·0 ...)	38·2
Biotin H	60·0 (Fair)	71·0 (Good ...	82·0 ...)	94·0	109·0 (Excellent)	83·2
Nicotinic acid	53·0 (Fair ...	61·0 ...	57·0 ...	51·0 ...	46·0 ...)	53·6
Control	43·0 (Fair ...	45·0 ...	46·0 ...	46·0 ...	44·0 ...)	44·8
Average	55·8	62·2	67·4	70·4	71·1	

SUMMARY OF DRY WEIGHT RESULTS.

	S. E.	C. D. at 1% level
Treatments	0·9	3·34
Vitamins	0·636	2·4
Concentrations	0·78	3·0

VITAMIN TREATMENTS

Vitamins	B_1	>	H	>	B_2	B_6	>	Nicotinic acid	>	Control	>	Ascorbic acid
Dry wt. in Mgs	94·4		83·2		73·4	72·2		53·6		44·8		38·2

CONCENTRATION TREATMENTS

Concentration in μg per litre	500	200	>	100	>	50	>	10
Dry wt. in Mgs.	71·1	70·0		67·4		62·2		55·8

TABLE 2

Showing average growth in Mgs. and sporulation of *Phyllosticta artocarpina* at different concentrations of the vitamins. The nature of sporulation is indicated in brackets.

Vitamins	Amount of vitamins in mgs. per litre					Average
	10	50	100	200	500	
Thiamin B_1	76·0	81·0	89·0	109·0	127·0	96·4
	(Excellent ...	...	... ...	... ...	... ...)	
Riboflavin B_2	61·0	69·0	76·0	81·0	88·0	75·0
	(Good ...	...	... ...)	... (Excellent)		
Pyridoxine B_6	65·0	71·0	79·0	91·0	102·0	81·6
	(Fair)	(Good ...	...	... ...	... ...)	
Ascorbic acid C	61·0	64·0	54·0	46·0	38·0	52·6
	(Fair ...	...)	(Poor ...	...	... ...)	
Biotin H	71·0	78·0	84·0	96·0	111·0	88·0
	(Fair)	(Good)	(Excellent	...	... ...)	
Nicotinic acid	65·0	69·0	73·0	67·0	51·0	63·0
	(Fair ...	...	... ...	... ...	... ...)	
Control	56·0	56·0	55·0	53·0	51·0	54·8
	(Fair ...	...	... ...	... ...	... ...)	
Average	65·0	69·7	72·8	77·5	81·5	

SUMMARY OF DRY WEIGHT RESULTS

		S. E.	C. D. of 1% level
Treatments	...	0·867	3·24
Vitamins	...	0·612	2·291
Concentrations	...	0·75	2·808

VITAMIN TREATMENTS

Vitamins	B_1	>	H	>	B_6	>	B_2	>	Nicotinic acid	>	
Dry wt. in mgs.	96·4		88·2		81·6		75·0		63·0		

Control	Ascorbic acid
54·3	52·6

CONCENTRATION TREATMENTS

Concentration	500	>	200	>	100	>	50	>	10
Dry wt. in mgs.	81·5		77·5		72·8		69·7		65·0

It is evident from tables 1 and 2 that action of different vitamins on *Phyllosticta cycadina* and *P. artocarpina* was not uniform and their were some minor differences amongst the two fungi. Thiamin (Vitamin B_1) gave significantly best growth of both the fungi. Growth of these organisms increased with the increase in the concentration of thiamin. Biotin (Vitamin H) was next best for promoting the growth of both the species of *Phyllosticta.* The best results were obtained at highest concentration of this vitamin also. *P. cycadina* showed significantly good growth on riboflavin (Vitamin B_2) and pyridoxine (Vitamin B_6) though higher concentrations of B_6 (more than 100 μg per litre) were not suitable for the growth of this organism. Pyridoxine gave better growth of *P. artocarpina* than riboflavin and unlike *P. cycadina* the growth of *P. artocarpina* increased with the concentration of pyridoxine. The growth of these two fungi was not very satisfactory on nicotinic acid. As in both cases it decreased when its concentration was above 50 μg and 100 μg per litre respectively. Both the organisms showed significantly poor growth on ascorbic acid (Vitamin C) and concentrations above 10 and 50 μg per litre were toxic for the growth of *P. cycadina* and *P. artocarpina* respectively.

The above two tables also indicate that sporulation of these fungi was also influenced by various vitamins and their concentrations, though both the species of *Phyllosticta* could sporulate easily even in complete absence of vitamins. Excellent sporulation of both the organisms was supported by thiamin. The sporulation of *P. cycadina* was fair only at 10 μg per litre of riboflavin, while its higher concentrations supported good sporulation of this fungus. Concentrations of riboflavin upto 100 μg per litre induced good sporulation of *P. artocarpina*, which became excellent by further increase of its concentration. Sporulation of *P. cycadina* was fair at 10 μg concentration of pyridoxine. It was good at 50 and 100 μg per litre and excellent at 200 and 500 μg per litre, while *P. artocarpina* gave good sporulation when concentration of this vitamin ranged between 50-500 μg per litre. Only fair sporulation of this organism was recorded at 10 μg per litre. Lower concentrations of ascorbic acid supported fair sporulation of both the fungi, but any increase in its concentration above 100 μg per litre for *P. cycadina* and 50 μg per litre for *P. artocarpina* decreased the sporulation. The sporulation of both the organisms increased (from fair to excellent) with increase in concentration of biotin. Nicotinic acid supported only fair sporulation of these fungi.

DISCUSSION

Hawker (1936), Leonian and Lilly (1938) and Fries (1948) have reported a number of organisms which require external supply of vitamins for their growth, but the present fungi could grow without external supply of vitamins. No doubt their addition, upto a cartain limit, was useful. Lewis (1952); Grewal (1954) and Agarwala (1955) found that thiamin or riboflavin were not necessary for the growth of *Alternaria solani, A. tenuis* and *Gloeosporium psidii* respectively, but addition of these vitamins markedly increased the growth of their fungi and in this respect they behaved like the present organisms. Growth of *P. artocarpina* increased with the increase in concentration of pyridoxine, but the growth of *P. cycadina* decreased at higher concentrations of this vitamin. Agarwala (1955) also mentioned that the growth of *Gloeosporium limetticolum* and *G. citricolum* increased at lower concentrations of this vitamin.

Grewal (1964) observed that increase in the amount of ascorbic acid (vitamins C) increased the growth of *Colletotrichum papayae.* The present results differed from those of Grewal (1954), as the growth of *P. cycadina* and *P. artocarpina* decreased when the concentration of this vitamin was more than 10 μg and 50 μg per litre respectively. Hawker (1939) has mentioned that certain strains of *Melanospora destruens* could grow in absence of biotin, but the mycelial growth increased when its concentration was increased. The fungi under investigation behaved in the same manner. Bekman et al (1953), Tremaine and Miller (1954), and Sadasivan and Subramanian (1954) also found that biotin was important for the growth of their fungi.

Neurospora sp and *Blastocladia* sp were found to be deficient for nicotinic acid by Bonner (1946) and Cantino (1948) respectively. The present fungi showed very slight improvement in their growth at lower concentrations of nicotinic acid but its higher concentrations were decidedly toxic for their growth.

The vitamins have also been reported to influence the sporulation of fungi. Hawker (1939) working with *Melanospora destruens* and Barnett and Lilly (1947) with *Sordaria fimicola* observed that their perithecial production could be controlled by the amount of thiamin. Grewal (1954), however, observed that increase in its concentration beyond 10 μg per litre did not increase sporulation any further. The present investigations established that *P. artocarpina* gave excellent sporulation even at lowest concentration of thiamin, while *P. cycadina* gave excellent sporulation at slightly higher cencentrations. Barnett and Lilly (1947) found that addition of biotin even after 42 days of growth greatly increased the development of perithecia of *Sordaria fimicola.* Grewal (1954) also recorded good sporulation of all the fungi investigated by him. Excellent sporulation of both the species of *Phyllosticta* was also supported at higher concentrations of this vitamin. Increase in concentration of riboflavin and pyridoxine also increased the sporulation of the present organisms, but the higher concentrations of ascorbic acid greatly decreased the sporulation of both the fungi.

Agarwala (1955) observed that sporulation of *Gloeosporium citricolum* increased with the increase in concentration of nicotinic acid. The present results differed from those of Agarwala (l. c) because the sporulation of both the species of *Phyllosticta* did not increase at higher concentration of nicotinic acid,

SUMMARY

The influence of various concentrations of six different vitamins on the growth and sporulation of *Phyllosticta cycadina* and *P. artocarpina* was studied. It was found that these fungi could easily grow and sporulate in absence of vitamins, but their addition accelerated the growth. Increase in the concentrations of thiamin, riboflavin and biotin greatly improved the growth of both the organisms. Higher concentrations of ascorbic acid and nicotinic acid decreased the growth of these fungi. The two species of *Phyllosticta* behaved differently on pyridoxine because higher concentrations of this vitamin could improve the growth of *P. artocarpina* even though they were toxic for *P. cycadina*. In general the vitamins which were good for growth were good for sporulation also.

ACKNOWLEDGEMENTS

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ON A NEW AVIAN TREMATODE OF THE GENUS BRACHYLAEMUS (DUJARDIN, 1843) BLANCHARD, 1847

By

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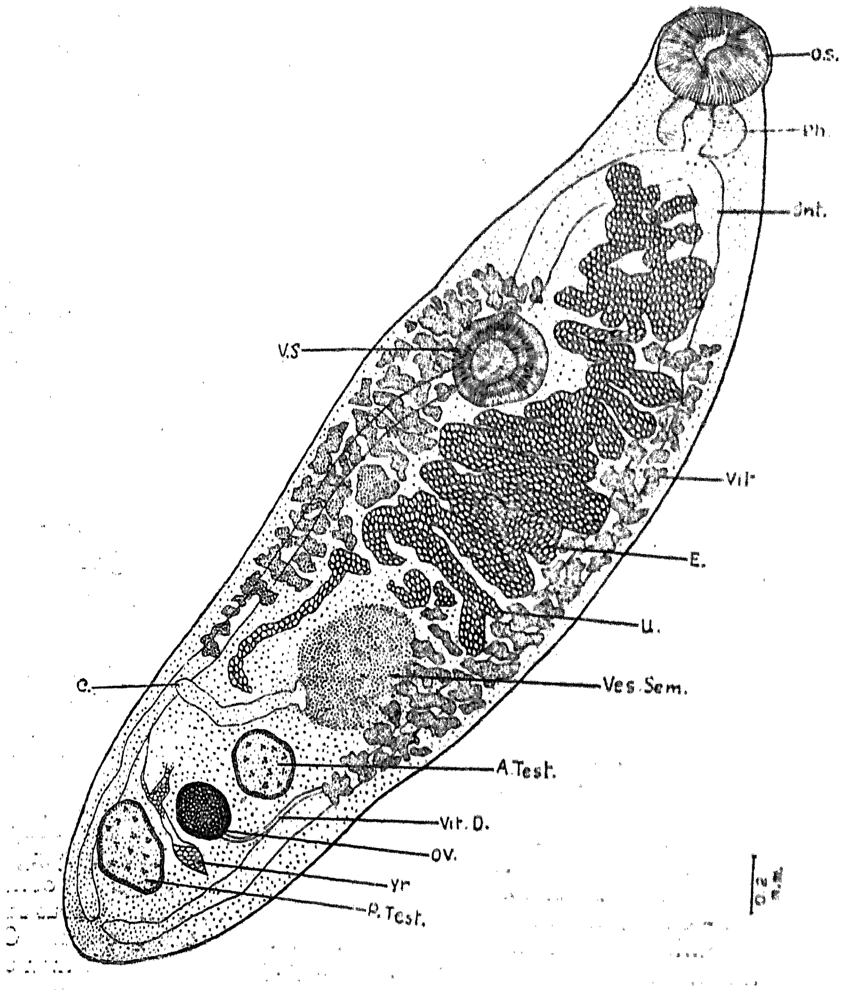
Brachylaemus (Dujardin, 1843) Blanchard, 1847.

Dujardin (1843) created the genus *Brachylaima* for the two species *B. advens* and *B. fulvus* Dujardin, 1843, and in 1845 he revised his own genus and changed the name to *Brachylaimus*. Blanchard (1947) finally modified and amended the genus and changed the name again to *Brachylaemus*. The genus *Harmostomum* Braun, 1899, was considered as a synonym of *Brachylaemus* (Dujardin, 1843) Blanchard, 1847. For the reception of this genus the sub-family *Brachylaeminae* and family *Brachylaemidae* were created by Jeyeux and Feley, 1930. Among the helminthologists who have worked on this genus and added new species to it the name of Baer (1923), Stiles and Stanley (1932), Dollfus (1934), Jeyeux, Baer & David (1934) and Sandground (1938) are worth mentioning. Among the recent workers Ulmer (1951) has revised this sub-family in the light of life histories. In this paper I have described a new species of *Brachylaemus.*

Brachylaemus tisa h. sp.

Two specimens of this brachylaemid were collected from the small intestine of one of the two kites, *Butaster tisa* shot at Cuttack in the month of January 1955. When fresh, the parasites showed a remarkable degree of contraction and elongation. Body tounge shaped,* 3·22 - 3·71 ×0·504 - 1·064 in size (maximum breadth immediately behind acetabular zone). Cuticle smooth. Oral sucker robust, spherical, terminal 0·266 - 0·322 ×0·322 - 0·336, with a narrow mouth opening. Pxarynx well developed, touching the oral sucker, 0·14 - 0·168 ×0·21 - 0·266; oesophagus absent, the intestinal caeca starting immediately from the pxarynx, running parallel to the body well right to the end of the body. Acetabulum spherical with a wide opening, 0·308 - 0·322 ×0·308 - 0·322, placed at 0·366 - 0·63 behind the intestinal bifurcation or at the junction of the first and second third of the body length. Gonads tandem, near the median line, in the posterior quarter of the body, the ovary being between the testes. Anterior testis spherical, 0·196 ×0·21, lying 1·260 behind the acetabulum. Posterior testis subspherical or elongated obliquely 0·238 - 0·28 ×0·196 - 0·224, at 0·21 - 0·238 in front of hind end. Cirrus sac not visible. Vesicula seminalis voluminous, just in front of the anterior testis. Cirrus very long, unarmed, protrusible, 0·5 ×0·084. Ovary spherical, smaller than testes, 0·154 ×0·182 - 0·196, at 0 084 behind the anterior testis. A receptaculum seminis, Laurer's canal and shell gland mass were not seen. Uterus voluminous, mostly intercaecal, its field extending from the anterior testis up to the intestinal bifurcation. Eggs numerous, golden yellow, and so highly refractile and closely packed that they look like a mass of fat globules. The genital pore is median, situated immediately in front of the anterior testis. The vitellaria consist of small follicles grouped laterally in the intercaecal, caecal and extracaecal fields from the anterior level of the acetabulum to that of the anterior testis. Transverse vitelline ducts unite, and dilate to form a conspicuous yolk reservoir, immediately behind the ovary.



Key to the lettering of the figure Brachylaemus tism n. sp.

E, Egg; C, Cirrus; Int, Intestine; Ov, Ovary; O.S., Oral sucker; A. Test, Anterior testis; P. Test, Posterior testis; Ph, Pharynx; U, Uterus; V.S., Ventral sucker; Vit, Vitellaria; Vit. D., Vitelline duct; Yr, yolk reservoir.

Taxonomic Discussion.

Brachylaemus tisa n. sp. resembles *B. fulvus* (Dujardin, 1843) Blanchard, 1847, in its topography, sub-equal suckers and in the absence of a prepharynx and oesophagus ; but it can be differentiated by the absence of cuticular spines and serrated body wall and intestinal wall and by its well developed uterine coils (poorly developed in *B. fulvus*) a well developed protrusible cirrus and the seperated gonads (gonads overlap each other in *B. fulvus*.)

Brachylaemus arcuatus (Dujardin, 1845) Blanchard, 1847, resembles *B. fulvus* and therefore can be differentiated from the new species by most of the characters mentioned above.

The Brachylaemus species described by Sandground (1938) comes very close to *B. helicis* (Meckel, 1846) Baer, 1928: but both these differ from the new species in having the acetabulum much larger than the oral sucker (sub-equal in *B. tisa* n. sp.) in the vitellaria never extending in front of the hind edge of the acetabulum (in the new species they extend beyond the front edge of the acetabulum), in the genital pore being placed over the anterior testis and in the absence of a long petrusible cirrus.

Host :—Kite, Butaster tisa.

Habitat :—Small intestine.

Locality :—Cuttack, Orissa, India.

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* All measurements are given in mm.

EFFECT OF MIXED CROPPING ON GROWTH BEHAVIOUR AND YIELD OF ASSOCIATE CROPS AND ON NODULATION OF COMPANION LEGUME

By

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The varied problems connected with mixed cropping in the growing of a legume in association with non-legume annuals led to the general enquiry whether such an association was useful, specially, in view of the several conflicting results reported on the influence of legume on the associated non-legume and *vice-versa*. Pilz. (1911), and Madhok (1940), among others, recorded better legume growth from pure stands whereas Lipman (1912), Stalling (1926), Ludwig and Allison (1935), Wagner and Wilkins (1947), Lyon and Bizzell (1911), Thoronton (1935) and others have shown mutual benefits of associated cultures.

The importance of optimum seed rate proportionality of crops, grown mixed, having been demonstrated for the most remunerative growth and yield, under field conditions (Singh, 1957), the next step taken was to evaluate the effect of interaction of crops in mixed cultures of wheat, gram and mustard, the widely used mixture association in varied combinations on the growth, composition and yield of the companion crops.

MATERIAL AND METHODS

A field experiment was laid out on unmanured sandy loam soil, typical of the Gangetic alluvium, with moderate fertility, after the simple randomized replicated deisgn: the field being divided into four blocks, each of which was subdivided into seven 1/40 acre plots to accommodate the seven crop combination variations: Wheat (W), gram (G), mustard (M), wheat + gram (WG), wheat + mustard (WM), mustard + gram(MG) and wheat + gram + mustard(WGM).

Acclimatized pure strain seeds of wheat (*Triticum vulgare* NP_{52}) gram (*Cicer arietinum*, NP_{87}) and mustard (*Brassica nigra*, NP_{80}) were choiced for their representative biochemic constitution and wide economic utility as food. The previously determined (Singh, 1957) optimum seed rate proportionality among the associates, on weight basis, was maintained as W:G :: 4:1, W:M :: 10:1, G:M :: 6:1 and W:G:M:: 20:5:1. Based on the prevalent seed rates, the crops and their assciates were raised with the quantity of seed, expressed as lb/acre, shown against each: W=50, G=30, M=5, WG=32+8, WM=25+2·5, GM=15+2·5 and WGM=30+7·5+1·5. Wheat and gram were sown behind the plough and mustard was evenly broadcasted, in between the rows, whenever required, to ensure equitable and homogeneous distribution.

Routine cultural operations viz., interculture, weeding, etc., as well as sampling and measurements, were done from a number of plots each day and the choice was made in a systematic manner in which the replicates and treatments were randomized.

Quantitative assessment of growth performance of plants in response to treatments was done through measurements, at regular intervals, of change in vertical growth, tiller production and assimilating leaf surface in the case of wheat; diameter of the spread, number of branches, nodule count, nodule weight, and dry matter accumulation of tops and of roots in gram; vertical growth, and dry matter accumulation in mustard. Developmental characters, such as length of ear, number of spikelets and of grains per ear, and number of headed tillers per plant in wheat along with yield of grain and straw of the crops was recorded. Leaf area was determined by the leaf product method as advocated by Miller (1938).

Protein Nitrogen: Separation of the non-protein nitrogen was accomplished by extracting repeatedly with small quantities of boiling water one gram sample of the finely ground air-dry tissue. The insoluble residue was collected on a filter paper, dried, weighed and transferred to a kjeldahl's flask. The kjeldahl method (A.O.A.C., 1945) was followed for the determination of nitrogen. Digestion was continued for an optimum period of six hours as recommended by Chibnal and associates (1943) and Miller and Houghton (1945).

EXPERIMENTAL RESULTS

WHEAT

Vertical Growth

Shoot elongation was significantly largest throughout the life cycle in the WGM mixture followed respectively by WG, W, and WM. Wheat, grown singly, grew tallest than in association with mustard at all growth stages except at tillering. Association with gram helped wheat to attain better growth in height as compared with W or WM treatments except at pre-earing stage (Fig. 1a). The association of mustard thus definitely retarded growth of wheat while that with gram was advantageous.

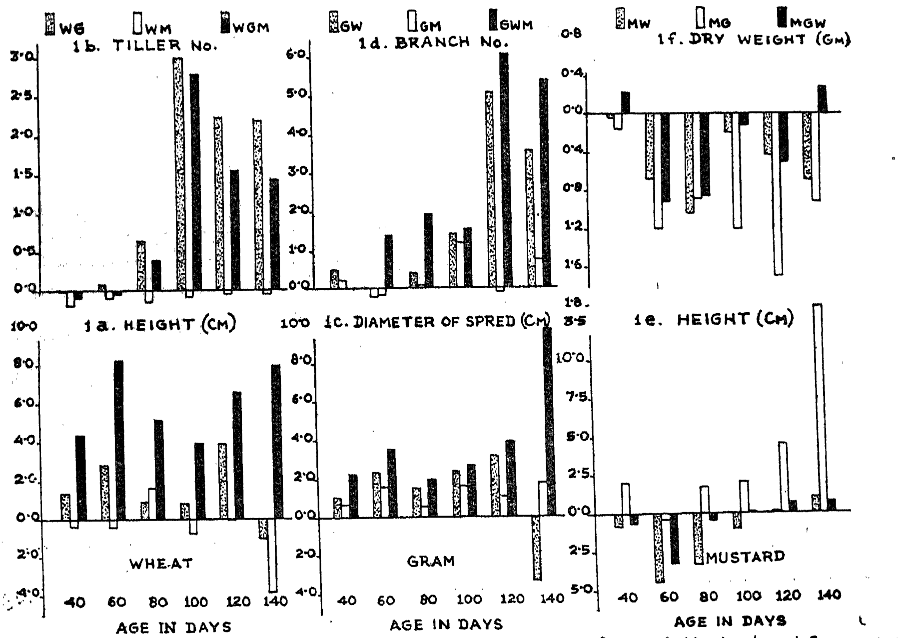

FIG.1. Effect of Crop Association on the growth behaviour of Wheat, Mustard and Gram over single culture of these.

Tillering

The association of mustard seemed to be of definite disadvantage to wheat; significantly lowest tillering was obtained in the WM association at all stages of growth (Fig. 1b). Tillering, however, increased significantly in association with the legume (WG); a more marked difference was noticed between WG and WGM than between W and WM, although tillering response was not significant in either case. Wheat responded to mixed cropping treatment as WG > WGM > W > WM in respect of tillering (Table 1).

TABLE 1

Effect of Mixed Cropping on the Wheat Plant

Growth characters	Crops	Age in days 40	60	80	100	120	140
Tillers	W	6·72	8·46	10·17	8·46	8·30	8·33
(average/	WG	6·71	8·54	10·82	11·47	10·54	10·54
plant)	WM	6·52	8·37	10·02	8·38	8·25	8·29
	WGM	6·62	8·41	10·56	11·28	9·88	9·75
		SE = 0·2839		CD = 0·8555			
Leaf area	W	65·50	105·60	115·73	145·34	129·69	54·73
(sq. cm/plant)	WG	69·34	118·34	132·51	161·23	138·31	61·85
	WM	63·11	104·29	109·43	138·80	124·17	50·03
	WGM	67·22	116·52	121·90	152·63	130·57	55·32
		SE = 1·6	CD = 5·82				

Judged in relation to age (the physiological stages of the cereal), tillering increased consistently upto pre-flowering in all combination treatments; with the onset of flowering, it declined in W and WM mixtures (Fig. 1b). In the WG and to a lesser degree in WGM cropping schemes, tillering capacity kept on an increase till the milky stage and the culms were maintained green for almost 10 days longer than control (W).

Assimilating Leaf Surface

WG increased leaf area of wheat significantly, WGM depressed it, to give it the second place, though significantly only in case of WG, WM combination induced minimum leaf area expansion. In WGM leaf area was between W and WG owing to the depressing effect of M in the combination (Table 1).

Considered in terms of age-leaf relation, the association of gram increased assimilating leaf surface even after the initiation of flowering of wheat unlike that in single culture, and WM, which exhibited sudden drop. The influence of legume

cereal companionship was, thus, clearly manifested in increased tillering upto milky grain stage, prolonged vertical growth, continued increase in leaf area even after flowering stage and an increase in the life cycle of the wheat plant by about 10 days, (Fig 1).

Headed Tillers

The number of ear-bearing tillers was significantly lowest in WM, WG producing maximum (Fig. 2a). Of W and WG, the mixture crop proved significantly superior in raising the number of headed tillers. Wheat plants of WGM association produced headed tillers in between that of W and WG, the treatments arranging as WG > WGM > W > WM in descending order. The differential effects in the response to raise the number of ears of associated growth were not disturbed by the age; in all treatments ears appeared between 80-100 days from germination.

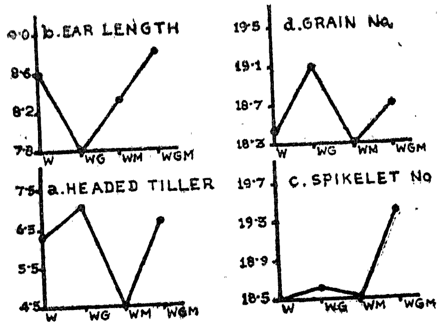

Fig. 2. Influence of Crop Association on Wheat

Linear Growth

WGM companionship produced significantly longest ears, W establishing a close run with WGM (Fig. 2b). Mustard companionship, very definitely, reduced ear length of wheat. Shortest ears in the WG series indicated that this partnership was not favourable. Though ear length increased with age of the plants the differential effect with different associates remained unaltered.

Spikelets per Ear

The triple mixture combination produced largest number of spikelets per ear, it decreased in the order of treatment combinations : WGM > WG > WM > W. No significant differential response was in sight between the treatments WG and WM on the one hand, and W and WM, on the other (Fig. 2c).

Grains per Ear

Significantly largest number of grains to the ear were produced in WG combination mixture, followed by that in WGM, W and WM in succession (Fig. 2d). Judging on the number of grains developed out of the spikelets present (cf. Figs. 2c, 2d) larger extent of fertilization appeared to occurr in WG as compared to WGM in which spikelets/grain ratio was higher.

Spread of Tops

Compared to G, the diameter of the spread increased significantly in GWM, GW and GM in the descending order (Fig. 1c). Growth in the from of the diameter of the spread of gram continued upto grain formation stage in GW while in control (monoculture of gram) and in GM combination the spread was arrested 2-3 weeks earlier.

Branching

GWM and GW combinations increased branching significantly in extent as well as number, the former being significantly superior. Minimum branching was recorded in G, WM did not show significant superiority (Fig. ld). Wheat-gram association was definitely advantageous whereas WM proved detrimental in magnitude of spread and branching, at successive stages. The association effect was significantly marked in GWM for maximum branching followed by GW, GM and G in succession.

Growth of Tops

The combination GM proved significantly better in dry matter in accumulation of tops than gram grown singly. Of the association, GWM produced the largest top dry matter at all the stages in the life cycle of the plant (Table 2). GW association proved better, insignificantly though, as against GM. Largest rate of dry matter accumulation was observed during 60-80 day period after germination followed by that in the pre-flowering (80-100 days period).

TABLE 2

Effect of Mixed Cropping on Dry Matter Accumulation in the Legume Companion

Growth characters	Crops	Age in days					
		40	60	80	100	120	140
Tops	G	1·702	2·437	3·717	4·653	4 403	4·260
(gm/plant)	GW	2·175	2·805	5·205	7·947	8·690	7·818
	GM	1·876	2·678	4·763	7·653	7·820	6·474
	GWM	5·427	7·934	11·070	13·245	15·629	14·825
			SE	0·5022	CD =	1·5132	
	G	0·5560	0·3623	0·5289	0·6448	0·6395	0·6275
Roots	GW	0·5908	0·7202	1·3268	1·8668	2·0737	1·9870
(gm/plant)	GM	0·3636	0·5528	0·9575	1·3518	1·7084	1·4618
	GWM	2·6567	2·1419	3·662 8	3·1282	3·9331	3·7900
			SE	0·1109	CD=	0·3342	

Root Growth

Root growth, as judged by dry matter formation, was affected by mixed cropping to the level of significance (Table 2). The decreasing response could be arranged as GWM > GW > GM > G, all the treatments differed significantly, GWM and GW being highly significant over control.

MUSTARD

Vertical Growth

Mustard attained significantly maximum vertical growth in the mixture combination MG followed by MGW, MW and M ; last three crop combinations failed to produce any significant difference amongst themselves. MGW combination proved deleterious for growth in height upto 80 days, neutral at 100 day, and afterwards advantageous. The beneficial effect of gram on the growth of mustard was visible although the associationship was not suggestive of distinct mutual benefit.

Dry Matter Accumulation

Grown singly, mustard showed maximum dry matter production, followed by MGW, MW and MG (fig. 1f). MG combination mixture significantly depressed dry matter synthesis indicating this companionship to be unfavourable.

EFFCT OF ASSOCIATED CULTURES ON THE PRODUCE OF COMPANION CROPS

Wheat

Significantly higher grain yield was produced by W or WG treatments as compared to WMG or WM (Table 3).

TABLE 3

Effect of Crop Association on Yield of Wheat

Yield	Replicates	Wheat combinations			
		W	WG	WM	WGM
Grain (md/acre)	I	16·097	14·949	11·943	12·876
	II	15·124	14·281	12·000	12·645
	III	17·368	15·856	12·374	12·862
	IV	17·004	16·600	11·582	12·597
		SE =0·313		CD =1·001	
Straw (md/acre)	I	28·567	35·479	21·701	26·005
	II	25·436	34·365	22·876	25·176
	III	30·289	36·274	22·940	25·578
	IV	30·046	36·542	20·529	25·876
		SE =1·949		CD =6·234	
Total (md/acre)	I	44·673	50·428	33·644	38·881
	II	40·560	55·130	34·876	37·821
	III	47·657	51·130	35·314	38·440
	IV	47·050	53·142	32·111	38·473
		SE =1·060		CD =3·390	

No significant differences in yield were in sight between W and WG on one hand, and WM and WGM on the other. Mixture WG increased straw yield significantly over other treatment combinations. Wheat, grown singly, produced straw significantly in excess of that obtained in WM indicating that mustard association also depressed straw yield (Table 3). The differences in yield of straw between WG and W, on one hand and WGM and WM on the other were not significant though the former in each case produced more.

Considered on the basis of total produce, the yield responses may be arranged as WG>W>WGM>WM in the descending order, the first two crop combinations being significantly and positively effective over WM (Table 3).

Gram

Monoculture of gram produced significantly maximum seed yield while GM and GW produced significantly more than the triple combination mixture. Thus gram in association led to reduction in seed yield (Table 4)

TABLE 4
Effect of Crop Association on Yield of Gram

Yield	Replicates	Gram combinations			
		G	GW	GM	GWM
Grain	I	14·185	12·812	13·118	8·829
(md/acre)	II	15·421	12·742	13·576	7·897
	III	14·286	12·615	12·629	8·740
	IV	14·379	13·074	13·237	9·238
		SE =0·2433		CD=0·7882	
Straw	I	7·026	10·267	9·434	16·467
(md/acre)	II	6·745	11·378	9·546	17·887
	III	7·000	10·223	10·029	16·562
	IV	7·978	11·026	8·958	15·479
		SE =0·3855		CD = 1·233	
Total	I	21·210	23·039	22·552	25·296
(Md/acre)	II	22·166	24·160	23·122	25·783
	III	21·286	22·838	22·658	22·302
	IV	22·357	24·100	22·195	24·717
		SE =0·3608		CD =1·154	

Straw yield responded to the several mixture treatments in the reverse order : GWM > GW > GM > G. Differences in straw yield between treatments GW and GM were significant, but less marked. Other mixture combinations brought about highly significant effects (Table 4).

The overall yield (seed + straw) of gram was significantly less when gram was grown alone than in combination. Maximum yield was obtained in GM combination mixture closely followed by GW, GM and G. GM failed to produce any significant increase over control. The yield difference between GWM, GW and GM were significant.

Mustard

Mustard, grown singly, produced maximum seed, increase over other treatments being highly significant. Of the combinations yield of seed in WM combination was greater than MG or MGW (Table No. 5). Significantly the lowest yield obtained was in the triple crop mixture. The relative unfavourable nature of the MG association in the production of seeds was again evident.

TABLE 5

Effect of Crop Association on Yield of Mustard

Yield	Replicates	Mustard combination			
		M	MG	MW	MGW
Grain (md/acre)	I	8·422	6·635	6·764	5·927
	II	9·368	6·126	6·879	5·379
	III	8·394	5·874	7·236	5·379
	IV	8·394	5·874	6·402	6·474
		SE	= 0·242	CD	= 0·774
Straw (md/acre)	I	10·132	13·369	8·570	10·058
	II	11·488	13·862	8·879	9·478
	III	9·449	13·981	9·823	9·656
	IV	10·002	12·605	8·214	9·968
		SE	= 0·3295	CD	= 1·054
Total (md/acre)	I	18·554	20·004	15·354	15·985
	II	20·856	19·988	16·758	14·714
	III	17·376	21·407	17·059	15·035
	IV	18·396	18·579	14·616	16·742
		SE	= 0·9551	CE	= 3·055

MG combination proved best for straw yield, pointing to the excellence of association of gram for vegetative growth (Table No. 5) as also noted in the case of wheat. Mustard, singly, gave promise of producing more straw than MGW. MW yielded mustard straw significantly less than M or MG. The wheat-mustard association was little beneficial. From the point of view of overall production of mustard (seed + straw) MW yielded largest, though significantly greater than MW and MGW treatments only and not M (Table 5). Tri-combination yielded less than MG but not MW.

Considered on unit area basis, the association of legumes and cereal components always yielded significantly more than single culture of either. The association of two or more crops tended to lower the seed yield of the components. A readjustment of straw/grain ratio appeared in associated growth producing more straw, that compensated the loss in grain yield and gave higher total yield on the account of each of the competing crops. It was specially true of gram.

EFFECT OF MIXED CROPPING ON PROTEIN CONTENT OF WHEAT AND GRAM SEEDS

Wheat grain produced from WG association showed significantly maximum percentage of protein followed by that in WGM, W, and WM mixture combinations (Table 6), but for the last two treatments significant differences were observed among others.

TABLE 6

Effect of Crop Association on Protein per cent of seed

Replicates	Wheat				Gram			
	Crop combinations				Crop combinations			
	W	WG	WM	WGM	G	GW	GM	GWM
I	12·210	13·164	12·026	12·804	18·514	18·670	18·648	18·619
II	12·214	13·160	12·000	12·802	18·506	18·676	18·650	13·620
III	12·209	13·162	12·027	12·810	18·512	18·674	18·643	18·621
IV	12·217	13·164	12·005	12·800	18·515	18·677	18·647	18·626
	SE = 0·00002		CD = 0·141		SE = 0·008		CD = 0·0256	

The percentage of protein in gram seed was also highest in the GW mixture combination to be followed by GM, GWM and G in succession. But for the mixture combinations GM and GWM, all the treatments depicted a consistent and significant rise, with age, in protein content.

Wheat and gram association, thus proved of mutual benefit in producing more grains of higher protein value. With the introduction of mustard, certain significant changes were noticed, specially in the lowering of protein percentage in the cereal-mustard association.

Nodulation in the legume was significantly enhanced numerically in the GW, GWM mixture as against other croping combinations. Grown mixed, no significant effect of mustard was observed on nodule counts in gram (Table 7).

TABLE 7

Effect of Mixed Cropping on Nodulation of Legume Companion

Nodule Character	Treatment	Age in days					
		40	60	80	100	120	140
Number ...	G	9·50	18·53	36·84	40·33	16·96	14·98
(Count/plant)	GW	12·02	29·46	48·34	53·18	27·37	20·14
	GM	11·19	20·43	40·42	42·07	19·63	16·31
	GWM	10·91	22·52	42·63	44·51	22·48	18·81
		SE=	0·7754	CD =	2·336		
Dry weight ...	G	23·75	32·50	61·25	46·15	32·60	30·50
(Mg/plant) ...	GW	42·45	73·12	91·37	59·45	44·02	40·95
	GM	36·35	37·37	61·75	47·90	33·30	31·37
	GWM	39·65	56·25	74·85	52·15	38·40	32·37
		SE =	2·547	CD =	7·675		

Judged by the dry weight of nodules, nodulation was affected favourably b mixed cropping in the descending order of officiency as GW > GWM > GM > G. Al treatments except GM showed significant effects in increasing the nodular mas over gram crop grown singly, while mustard as companion was found to be conduciv to poor nodulation in gram. The dry weight of nodules was on the increase fc eighty days irrespective of treatment combinations after which a slow decline wa recorded (Table 7.)

Plant Density

Crop association influenced plant population profoundly (Table 8.) Pla population was maximum when the crops were grown singly; with the introducti of a competing member, the number in unit area, fell for each crop though t total strength of individuals increased.

TABLE 8

Average Number of plants per acre

Crops	Crop treatments						
	W	G	M	WG	WM	MG	WGM
Wheat ...	60711	...	...	48460	49005	...	36213
Gram ...	...	60023	...	48216	...	56628	37853
Mustard ...	...	...	50910	...	46554	47371	34848
Mean ...	60711	60023	50910	48338	47779	51999	36305

In WG and WM combinations, the number of wheat plants was more than gram and mustard plants. In the MG mixture the number of the legume plants was in excess of mustard. In triple combination the number of gram plants was highest followed by wheat, and mustard in succession. Taking the total population of individuals in respect of mixtures grown the crop treatment arranged themselves in the descending order as W, G, MG, M, WG, WM, WGM.

DISCUSSION

The legume gram, when grown mixed with cereal wheat, benefitted in all respect except seed yield (Table 9). In the case of seed protein, the benefit due to the association of wheat or wheat-mustard was greater than that with mustard alone (Table 6).

TABLE 9

Overall Effect of Crop Associations on Legume Companion
(% increase or decrease over control)

Growth attributes	Associations					
	Gram, Wheat		Gram, Wheat & Mustard		Gram, Mustard	
Seed yield ...	(-)	12·07	(-)	40·42	(-)	9·81
Straw yield ...	+	49·09	+	130·87	+	32·54
Total yield ...	+	8·13	+	16·17	+	3·99
Nodule number ...	+	39·10	+	18·02	+	9·40
Nodule weight ...	+	54·96	+	29·53	+	9·39
Dry matter accumulation (tops).	+	63·98	+	221·76	+	44·82
Dry matter accumulation (roots).	+	180·03	+	491·07	+	109·10
Seed protein ...	+	0·86	+	0·59	+	0·70
Diameter of spread of tops	+	21·90	+	30·95	+	8·51

Gram plant gained in dry matter accumulation of roots, and tops, final straw yield, total produce and diameter of the spread, in diminishing degree in the associations GWM, GW, and GM as against its monoculture. In nodular mass, nodule number and seed protein the order of benefit to the gram plant was as GW > GWM > GM combinations. The triple combination, evidently, led to greater vegetative growth while in regard to nodule formation the wheat-gram association proved favourable. The mustard plant in association with gram kept the growth of the legume as well as the nodulation at a low level, though not lower than the lone crop of gram. Judging broadly, the effect of any association is measurable on the basis of the seed yield; the greater the loss in seed yield, as affected by the mixture combination, the greater was its usefulness with respect to growth and development of the legume although this did not apply to nodulation. In the case of nodulation some factor other than the mere regulation of the transfer of nitrogen from the legume to the soil owing to crop association seemed to control it, Seed rate proportionality between the crop mixture and the inter-penetration of the roots seem to be the factors.

With the introduction of companion crop, the growth of the components assumed special significance. As apart from single crop of either a legume or non-legume, in mixed cropping, legumes were shown to benefit the growth, development and dry matter production of the non-legume companion in general possibly resulting out of the ready availability of needed nitrogen (Singh, 1954). This was further supported by the gain in leaf area of wheat in the WG mixture combination since leaf area is known to be directly associated with nitrogen supply, other factors nonlimiting.

Root growth and nodulation were found to be benefitted by mixed culture of gram with any of the associate groups, wheat or wheat plus mustard. Evidence was put in before that yield of gram seed was significantly lowered when grown in association with either wheat or wheat plus mustard, the loss in seed yield being made up by the gain in straw. These findings lent further support to the view that conditions promoting prolific vegetative growth and high top/root ratio were not conducive to nodulation.

That cultural conditions like mixed cropping controlled the extent and nature of root excretions is quite clear from the results presented. It is likely that the plant roots secreted and supplied some substance to the microorganic population to help them promote growth. Larger excretions of nitrogen have already been demonstrated by Singh (1954). The findings are akin to those of Virtanen, West, Lundegardh and others who reported such excretions by roots. Virtanen (1938) reported that pea roots often excreted amino acids, aspartic acid and beta-alanine. West (1939) also showed that roots of flax and tobacco excreted growth factors such as aneurine and biotin. Similarly Lundegardh *et al* (1944) demonstrated that wheat roots could excrete nucloeotides and flavanones. Seed rate, crop rotation, mixed cropping green manuring and other cultural operations may thus, be used for adjustments of soil conditions and plant growth. Inducing proper distribution, ramification as well as functioning of crop roots may be achieved by correct crop associations, such that, nutrients needed for better growth and development of the crop may be increased for larger returns.

The most important biological feature in the whole field of symbiotic fixation of nitrogen is the nodulation in legumes. Nodulation is enhanced numerically and significantly when grown with wheat alone or wheat plus mustard as against other associations. The size, number, and weight of nodules depended on the proximity of the roots of the crops grown together; with the presence of a cereal alongside these attributes are on an increase, while the presence of mustard roots evaded the proper

growth, development and functioning of nodules. The effect of plant growth and its stage of development is also of immense value such that correct stage for cutting of grass mixtures for maximum benefit in the quality and quantity of output as well as soil amelioration varies with the mixture type and could be governed.

It is shown that one crop association may have its effect changed as soon as another crop is introduced even if the newly added crop may not show beneficial effect in the company of either of the crops in the original asssociation. This explains the distribution of plant species in different populations in nature to certain extent.

The amount of protein in plant also varied with crop mixtures The protein content of wheat could be raised by frequent small applications of combined nitrogen, which obviously affects the nitrogen metabolism (Singh, 1942). It is of importance to create conditions in which maximum value of protein may be stored in the seed so necessary to produce high quality of flour for bread making purposes and thereby the quality and market value of the seed.

When grown mixed, the protein value of the cereal as well as legume seeds increases and, thus, in the correct partnership with respect to crops and seed rate mixed cropping is of great economic advantage. Lyon and Bizzell (1911) reported similar increase in the protein content of the cereal grain when grown in association with legumes. Wagner and Wilkins (1947) obtained higher protein percentage in grasses when grown in association with legumes that excreted nitrogen. In another series of investigations, a supply of nitrogen was shown to increase the protein content of wheat grain grown under monoculture conditions (Singh, 1942) findings that served as an indirect evidence of nitrogen addition through the legume. Direct evidence of nitrogen excretition by the legumes has been afforded in a separate communication (Singh, 1954).

In the production of high protein wheat reduction in grain yield is accompanied if nitrogen availability is reduced. Both soil nitrogen and soil moisture that rank high in the obtaining of better quality and higher grain yield could be balanced and controlled through mixed culture *viz.* proper WG association.

Invariably the yield of the non-legume grain was depressed by the presence of the legume alongside, irrespective of the large significant gains in vegetative growth; wheat grain yield from associated mixtures of wheat plus gram was found to be less than wheat grown singly. Similar findings have also been reported by Wiggans (1937), Akers and Westover (1934), Fred and associates (1932), Brown (1935), Raju (1936) and Papadakis (1939), working with different mixtures. The loss in grain yield of the non-legume was shown to be amply compensated by the gain in yield of straw of the cereal and also of legume companion. Helm (1924), Raju (1936), Brown (1935) and Wiggans (1937) also reported similar performance. The general conclusion, thus, became obvious that the association of two crops need not be assumed to be harmful specially if their root systems tap from different layers of soil, or if the soil moisture and nutrients be not limiting.

In general, thus, the acre yield of seed of any individual crop may not be increased merely because it has been sown in association although the total output per acre may be considerably increased. Murneek (1937) and others cited examples where minute temperature changes induced alterations in the physiology of the plants. The data presented seem to indicate that this view could not be utterly disregarded as it is highly likely that the effect of sowing mixed would have been to effect changes in the physiology of the plant, occasioned by shading effects, as evidenced by greater vegetative growth and lesser grain output.

Legume growth was best in WG particularly with respect to the grain yield, the grain/ straw ratio of the produce was also high. It has been demonstrated, elsewhere, by the author that the grain/straw ratio was a function of the seed rate proportionality in the mixture; with increase in the seed rate of gram, the grain/straw ratio decreased (Singh,1957).

That the cereal grew better in association while the legume did not stand to loose has been amply demonstrated in these investigations. This characteristic nature of mutual benefit in associated cultures may be due to the ability of the legume to arrest (fix) more nitrogen symbiotically and also excrete it to the benefit of the non-legume (Singh, 1951). In explanation to such a behaviour under the stress of environmental conditions it seemed plausible that carbohydrate synthesis could not keep pace with nitrogen fixation and the excess of accumulated nitrogen in the nodule was made available to be taken up by the neighbouring absorptive root surface of the cereal wheat with lower nitrogen content.

The change in the yield responses to treatment mixtures in respect of steed and straw as giving quite reverse picture may possibly be attributable to a disturbance in the balance between the vegetative and reproductive phases, created by the introduction of the companion crop or crops, this view gained support in the change of ratio of straw: grain from 1:2 for single culture (G) 2·5:1, generally, for associated culture (cf. Table 4).

That total yield (grain+straw) of the composite mixtures, per unit area, speaks of the effects of the components from an economic stand-point.. Wheat, grown alone, seemed to give highest returns (44·985 md/ acre) through not significant over WG mixture (38·490 md/ acre) followed by WGM with 26·157 md to an acre. The other treatments stood in the decreasing returns as WM (24·840 md), G (21·755 md), MG (21·313 md), M (18·795 md) to the acre (Table 10). Here the gains in nitrogen by the soil and produce have not been taken into consideration and the total benefit for any combination could not thus be assessed, merely on the basis of production.

On a comparison of the outturn from the plots holding the cereal, legume and the oily crops singly or in association, it was made evident that the overall yield arranged themselves in groups, the first, principally composed of wheat and gram, in definite economic superiority, over the other group, principally dominated by mustard.

TABLE 10

Association Effect on Total Produce* (md/acre)

Plot No.	Crop mixtures						
	Wheat	Gram	Mustard	Wheat Gram	Wheat Mustard	Gram Mustard	Wheat-Gram Mustard
I	44·985	21·755	18·795	38·490	15·695	19·995	15·544
II	44·985	21·755	18·795	23·534	28·840	22·632	24·525
III	44·985	21·755	18·795	53·458	33·986	21·313	38·404
Mean	44·985	21·755	18·795	38·490	24·840	21·313	26·157

SE = 0·4076 CD = 6·7141

* Computed from tables 3, 4 and 5 on the basis of observed total produce of crops raised in an unit area.

It is important to note that, grown singly, wheat produced maximum, while mustard least; between these two limits lay rest of treatment combinations. Striking response was that while WG association produced consistently and significantly high, that due to MG proved depressant. The deleterious effect of mustard was very evident. Prevelant low seed rate of mustard in mixed cropping might be explained on the basis that in order to offset the detrimental effects of mustard the seed rate is kept very low, where the seed rate of mustard is increased high significant decrease in yield of other associate was certain. It would, thus, not be economically sound to sow mustard in mixed cultures where the practice of single culture may prove better.

SUMMARY

Varied problems connected with mixed cropping in the growing of a legume in association with other crop plants have been discussed. Statistical field trial was made on the effect of associated culture of wheat, gram and mustard grown in seven possible variations with pre-determined optimum seed rate proportionality.

Quantitative assessment of the effect of interaction of crops in mixed cultures on the performance of the plants in response to combination treatments was done at regular intervals. Records were taken of change in vertical growth, tiller production, assimilating leaf surface, length of the ear, number of spikelets and of grains per ear and the number of headed tillers in wheat; diameter of spread, number of branches, nodule count, nodule weight, dry matter accumulation of tops and of roots in gram; dry matter production and vertical growth in mustard. The protein content of wheat and gram seed and the final yield of the crops was also recorded.

Crop association was shown to influence plant population to a considerable extent. Largest number of plants were found when each of the components when, gram and mustard grew singly; with the introduction of competition in associated culture the number fell for each crop separately, while the total strength of individuals in unit area increased. The crop treatments may be arranged in the descending order as W, G, MG, M, WG, WM, WGM, in this behalf.

In the growth of wheat the crop combination WGM proved best for height, length of the ear and number of spikelets per ear while WG increased tillering, leaf area, heading, number of grains per ear and the protein content of seed significantly.

The growth of gram was stimulated by the association of wheat in respect of its spread, nodulation and protein content. GWM combination proved significantly best for branching, and extent of the growth of tops and roots.

For mustard, association with gram proved advantageous only in vertical growth, in dry matter production mustard grown alone gave best performance.

With the introduction of another crop the associated growth of the components assumed special significance. In mixed cropping, legumes were shown to benefit in respect of growth, development, dry matter production and protein content of the cereal companion. The legume benefitted in all respects except seed yield in association with cereal. In the case of seed protein, the benefit due to the association of wheat or wheat-mustard was greater than that of mustard alone.

Grown singly, wheat produced maximum, while mustard least; between these two limits lay rest of treatment combinations; the most striking difference in yield response was that while WG association produced consistently and significantly high, that due to MG proved depressant. The deleterious effect of mustard was well evident.

Overall yields arranged themselves in groups, the first, principally composed of wheat and gram, in definite economic superiority over the other group, principally dominated by mustard. The available data seem to indicate that the effect of sowing mixed would have been to effect changes in the physiology of the plant as evidenced by greater vegetative growth and lesser grain output.

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THE ASSIMILATION OF SUGARS BY THREE PATHOGENIC SPECIES OF PHYLLOSTICTA

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In Nature carbon is mostly available in complex form, but generally fungi convert them into simpler water soluble sugars of low molecular weight before utilization. The work of previous investigators on the role of various types of sugars on Physiology of fungi has been summarized by Kendrick and Walker (1938), Ajello (1948), Buston and Basu (1948), Hawker (1950), Lilly and Barnett (1953), Wolf (1953) and Bilgrami (1956).

Recent advances in chromatography have made it possible for plant physiologists to study the metabolism of sugars on the life of higher plants, but comparatively little work on these lines has been done with fungi. The authors have obtained some interesting results with the help of this technique. Their investigations as well as those of Bealing and Bacon (1953), Giri and Nigam (1954) and Roberts and McFarren (1954) have clearly established that the fungi could not only hydrolyse higher saccharides but they were also capable of synthesizing complex sugars from mono or di-saccharides. Such studies not only help to determine the process of assimilation of higher sugars by fungi, but may also throw light on new types of oligosaccharides.

The purpose of the present work is to study the utilization of six oligosaccharides and their hydrolytic products by *Phyllosticta mortoni* *P. morifolia* and *P. carica-papayae*. The effect of D. Mannose (which is closely related to D-Glucose and D-Fructose) have also been examined.

MATERIALS AND METHODS

Three species of Phyllosticta viz.; *P. mortoni* FAIR, *P. morifolia* PASS and *P. carica papayae* ALLESCH were isolated from the diseased leaves of *Mangifera indica, Morus alba* and *Carica papaya* respectively. The method used by Tandon and Bilgrami (1954) was followed for isolation and purification of the organisms. Different sugars were added individually to the basal medium* at a rate which supplied 4000 Mgms. of carbon per litre. Only A. R. grade reagents were used. 150 ml. conical Pyrex flasks were used for growing the organisms. Each flask contained 25 ml. of medium which was fractionally sterilized by steaming for half an hour daily for three successive days. Solutions containing these sugars were inoculated daily for 15 days at a fixed time (± 15 mts) with different species of Phyllosticta by Garrett's (1936) agar disc method. On the 16th day, the fungal mats were filtered separately from each set, when one to 15 days old cultures were

*KNO_3· 3·5 gms, KH_2PO_4 1·75 gms, $MgSO_4 . 7H_2O$ 0·75 gms. distilled water 1 litre.

available from different sets. The fungal mats of 6th, 11th, and 16th days served for quantitative measurements of growth. They were dried and weighed by the method described by Tandon and Bilgrami (1954). The filtrate of each day containing different oligosaccharides or their hydrolytic products was chromatographically analysed to detect the presence of various sugars. The circular paper chromatographic method described by Ranjan et al (1955) was used for this purpose. n-butanol-acetic acid-water (4 : 1 : 5) served as developing solvent, except in the experiments which contained raffinose, lactose, melibiose or their hydrolytic products, where the chromatograms were run in n-butanol-pyridine-water (6 : 4: 3). This was done to avoid the overlapping of the bands of glucose and galactose (as they gave a common band with the former solvent). A mixture of aniline-diphenyl amine phosphate (5 volumes of 4% aniline, 5 volumes of 4% diphenyl amine and 1 volume of phosphoric acid) was used for spraying the chromatograms. The other details of this method were similar to those used by Tandon and Bilgrami (1957). The average Rf values of various sugars were calculated and they have been recorded at appropriate places in the text.

EXPERIMENTAL

The amount of growth of three species of *Phyllosticta* in mgs. on raffinose, sucrose, maltose, cellobiose, melibiose, lactose and a mixture of their hydrolytic products is recorded in table 1.

TABLE No. 1

Showing dry weight in mgs. of three species of *Phyllosticta* on six oligosaccharides and their hydrolytic products.

(Each dry weight is average of three replicates)

		ORGANISMS		
SUGARS	DAYS OF INCULATION	*P. mortoni* Dry wt.	*P. morifolia* Dry wt.	*P. carica papayae* Dry wt.
Raffinose	5	12	8	10
	10	32	22	26
	15	40	30	34
Mixture of glucose, galactose & fructose	5	42	32	26
	10	76	58	42
	15	98	70	56
Maltose	5	22	20	58
	10	86	78	120
	15	120	98	137
Cellobiose	5	8	12	18
	10	26	24	30
	15	36	32	42
Glucose	5	54	42	52
	10	92	76	96
	15	110	92	130

SUGARS	DAYS OF INCULATION	*P. mortoni* Dry wt.	*P. morifolia* Dry wt.	*P. carica papayae* Dry wt.
Melibiose	5	18	13	22
	10	54	28	80
	15	86	36	104
Lactose	5	14	14	14
	10	26	26	30
	15	32	38	44
Mixture of glucose and galactose	5	56	40	52
	10	88	68	90
	15	116	90	118
Sucrose	5	44	18	48
	10	76	56	92
	15	98	74	106
Mixture of glucose and fructose	5	46	34	50
	10	72	64	90
	15	94	82	109
Fructose	5	42	36	40
	10	68	64	78
	15	88	78	101
Galactose	5	58	42	56
	10	88	72	98
	15	107	89	122
Mannose	5	36	34	44
	10	60	62	76
	15	78	76	92

Raffinose (Rf. 0·40). This trisaccharide on hydrolysis breaks as follows :

Raffinose (trisaccharide) → Sucrose → Fructose, Glucose

Raffinose (trisaccharide) → Melibiose (disaccharide) → Glucose, Galactose

(monosaccharide)

The dry weight results showed that this sugar was a poorer source of carbon than a mixture of its hydrolytic products. Chromatographic analysis of the medium containing raffinose showed that this sugar was not hydrolysed at any stage and could not be consumed completely by any of the organisms even at the end of the incubations period. An analysis of the mixture of the hydrolytic products revealed that *P. mortoni* and *P. carica papayae* assimilated both glucose and galactose in 7 days and fructose in 10 days, but *P. morifolia* was a little slower in utilizing these

sugars and it assimilated glucose, galactose and fructose in 9,10 and 12 day respectively.

Maltose (Rf. 0·55) and Cellobiose (Rf. 0·65). Both these sugars liberate two molecules of glucose on complete hydrolysis. They differ only in the pattern of the glycosidic linkage which unites the two residues of glucose. A glance of table 1 makes it clear that inspite of the fact that these two sugars differ only in the manner of glycosidic linkage, their availabilty to the three species of *Phyllosticta* varies considerably. This behaviour can be explained on the basis of the chromatographic results because maltose, which was the best sugar, was used through hydrolytic pathway, while cellobiose could not be hydrolysed by any of the organisms at any stage. Chromatographic results also showed the formation of an oligosaccharide (maltotriose Rf. 0·20) along with the hydrolysis of maltose. These results further indicated that *P. carica papayae* was quickest in hydrolysing maltose as in this case the traces of glucose were evident in the medium from the second day and it persisted in the medium upto 9th day. Maltose was also detected in the culture medium till the 7th day and maltotrise (the synthetic product) was seen upto 6th day only. The remaining two fungi viz., *P. mortoni* and *P. morifolia* appeared to be slow in producing maltose because in both cases glucose was detected on the 5th day and it persisted till the 10th day. The presence of maltotriose in case of *P. mortoni* and *P. morifolia* was from 5th to 9th and 5th to 10th day respectively.

Poor growth of *P. mortoni* and *P. morifolia* till 6th day may be correlated with delayed or slow activity of maltase after which the dry weight results show much rapid growth between 5th and the 10th day. (vide Table No. 1)

The identical oligosaccharide (cellobiose) did not exhibit any sign of break down and was slowly consumed by *P. carica papayae* in 13 days, while *P. mortoni* and *P. morifolia* could not finish this sugar even in 15 days. Chromatographic results seem to indicate direct assimilation of cellobiose by these organisms. Smith (1949) also suspected direct utilization of this sugar by *Marasmius chordalis*.

Lactose (Rf. 0·52) and *Melibiose* (Rf. 0·44)

These sugars liberate a mixture of glucose and galactose on acid or enzymic hydrolysis. The chromatographic results showed that the present organisms assimilated lactose through a non hydrolytic pathway, as no trace of any of its hydrolytic products was noticed in the culture medium. This sugar was not completely assimilated by these fungi even in 15 days. *P. morifolia* used melibiose also through a nonhydrolytic pathway and was incapable of finishing it completely but *P. mortoni* and *P. carica papayae* used it (melibiose) through a hydrolytic pathway due to which both glucose and galactose appeared in the culture medium from the 7th day *P. carica papayae* assimilated both the hydrolytic products in 10 days, while *P. mortoni* finished it in 12 days. Melibiose was also present, in both the cases, till 10th and 12th day respectively. Chromatographic studies revealed that these organisms synthesized an oligosaccharide* (Rf. 0·37) from melibiose. This oligosaccharide was produced by these fungi on the 7th day and it also remained in the medium as long as melibiose was not finished. Very little dry weight of these three fungi was attained till 6th day, but a rapid acceleration of growth was recorded between 6th and 11th day. The results from the table clearly show that lactose was a poor source for the fungi under study, while melibiose was found to be unsatisfactory for *P. morifolia* only (this organism was incapable of hydrolysing this sugar). The

* The exact nature of this sugar has not been established so far.

results further showed that a mixture of glucose and galactose was better utilized than lactose.

Sucrose (Rf. 0·60). This sugar served as a good carbon source for all the present organisms. Chromatographic studies showed that *P. carica papayae* entirely converted this sugar into a mixture of its hydrolytic products (glucose and fructose) within 24 hours of inoculation. *P. mortoni* caused complete convertion of sucrose in two days, while the hydrolysing capacity of *P. morifolia* was still slower as sucrose was present in the medium till 7th day. These results established that sucrose was utilized through a hydrolytic pathway. The chromatographic studies also showed that the fungi under study utilized glucose better than fructose, because it (glucose) was entirely used up in six days by *P. carica papayae*, in 7 days by *P. mortoni* and in 12 days by *P. morifolia*. The first two organisms utilized fructose in 10 days while *P. morifolia* finished it in 13 days. An identical pattern of growth of these fungi was seen on sucrose as well as on a mixture of its hydrolytic products, except for the fact that till 6th day *P. morifolia* showed much less growth on sucrose than on a mixture of glucose and fructose.

ASSIMILATION OF MONOSACCHARIDES (HEXOSES)

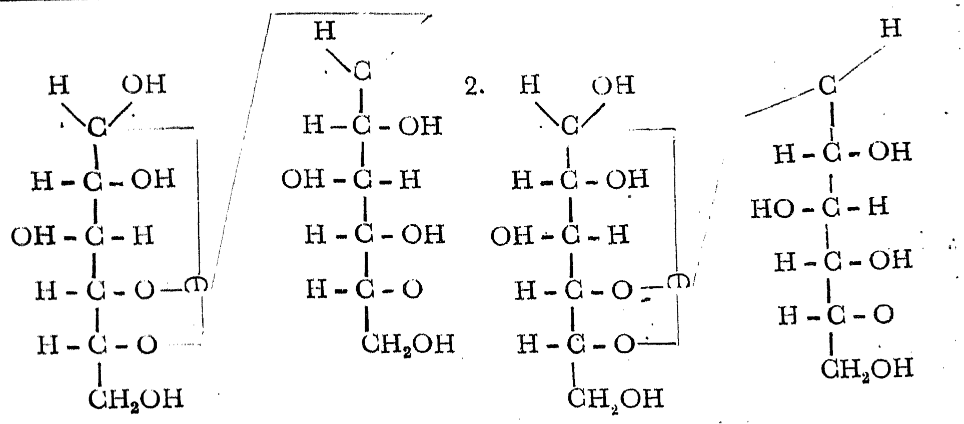
A further review of table No. 1 shows that out of the four hexoses used in these studies, glucose and galactose were very good sources for all the present organisms. Fructose and mannose were slightly inferior sources. Amongst these hexoses mannose supported least growth of *P. mortoni* and *P. carica papayae*, while fructose was found to be poorest hexose for *P. morifolia*.

DICUSSION

The present investigations clearly show that availability of oligosaccharides to the fungi depends on the production of suitable hydrolytic enzyme. Raffinose was an unsuitable source, while a mixture of its hydrolytic products was well utilized. *P. carica papayae* exhibited interesting results on break down products of raffinose. It was observed that the hydrolytic products individually or in combination of glucose with fructose or galactose served as much better source than a mixture of all the three hydrolytic products (viz., glucose, galactose and fructose). It is difficult to account for this behaviour of *P. carica papayae* because all the components of the mixture were taken up from the medium within 10 days of inoculation. Lilly and Barnett (1953) made similar observations for *Gliomastix convoluta* but they too could not find any explanation for this behaviour, because all the mono and disaccharides present in raffinose molecule were satisfactorily used. The present species of *Phyllosticta* differed from *P cycadina* (Bilgrami 1956) which was capable of hydrolysing raffinose.

The manner of glycosidic linkage in the structure of maltose and cellobiose has a pronounced effect on their suitability to the present fungi. Maltose in which the two glucose residues have *alpha* glycosidic linkage is easily hydrolysed (due to maltase activity), while cellobiose[2] which has *beta* glycosidic linkage is not hydrolysed. Lilly and Barnett (1953) reported that *Sordaria fimicola* and *Schizothecium longicolle* utilized cellobiose nearly as rapidly as glucose and on this basis they suggested that

cellobiose was probably hydrolysed before assimilation by an adaptive enzyme. The results obtained with the present fungi were different from those of Lilly and

1. 

Maltose   Cellobiose

Barnett, as no trace of glucose was detected during the assimilation of cellobiose and also because it was a poorer source than its hydrolytic product (viz. glucose). The previous results, about the nature of lactose were further confirmed as it was found to be a poor source for all the present organisms. It is generally considered that lactose is a poor source because galactose is unsuitable, but this does not appear to hold good for the species of *Phyllosticta* because galactose has been found to be satisfactory both individually and in combination with other sugars. Non-production of hydrolytic enzyme (lactase) appears to be the limiting factor. The results also revealed that these organisms synthesized maltotriose from maltose. *P. carica papayae* and *P. mortoni* also synthesized an oligosaccharide of unknown nature when they were grown on melibiose. It was also found that these oligosaccharides were only synthesized till both the hydrolytic product as well as the original disaccharides were available in the culture medium. The part played by the synthetic sugar is not clearly known but it is felt that they may be reconverted into original disaccharide, which in its turn is again hydrolysed before assimilation.

The invertase activity of these fungi differed because sucrose was hydrolysed with varying speed and the rate of assimilation of this sugar was dependent on its rate of inversion.

The results of this experiment showed that these fungi grew approximately at the same time and rate on galactose and glucose.

Hawker (1939) found that *Melanospora destruens* was totally unable to utilize galactose. Lilly and Barnett (1951) also found it to be an unsatisfactory source for many fungi studied by them. They considered that poor response of galactose was due to its structural configuration. Galactose differs from other three hexoses (viz., glucose, fructose, and mannose) in the arrangement of its 4th carbon atom. Srivastava (1955) established that it (galactose) was not only a poor source for *Alternaria tenuis* but the growth started later on galactose than on glucose. He explained that A. *tenuis* was unable to adapt itself to galactose because this sugar was of rare occurrance in plants, and the delayed response of this fungus to galactose, was due to the time taken by the organism to adapt itself to galactose. In the present

investigation the growth on galactose was not delayed and in this respect the results differed from those of Srivastava (1955). Chromatographic analysis of the leaves of the hosts confirmed the absence of galactose in all of them but inspite of this, these organisms did not need any time to adapt themselves to galactose and distinct growth was visible from the second day. The results further showed that structural configuration of galactose had nothing to do with the rate or amount of growth of these fungi. It is also interesting that fructose and mannose which were closer to glucose in structure, were inferior sources than galactose.

SUMMARY

Three parasitic species of *Phyllosticta* viz., *P. carica papayae*, *P. mortoni*, and *P. morifolia* were isolated from infected leaves of *Carica papayae*, *Mangifera indica* and *Morus alba* respectively.

The pathway of assimilation of six oligosaccharides by these organisms was studied chromatographically. Maltose and sucrose were assimilated through a hydrolytic pathway. Maltose was a better source than its hydrolytic product (glucose), while sucrose and a mixture of glucose and fructose were almost identical sources. Melibiose was hydrolysed by two species only viz., *P. carica papayae* and *P. mortoni.*

Raffinose, lactose and cellobiose were unsuitable sources and they were used up through a non-hydrolytic pathway. Hydrolytic products of these oligosaccharides supported very good growth of all the three fungi.

The organisms synthesized an oligosaccharide (maltotriose) from maltose. *P. carica papayae* and *P. mortoni* were capable of synthesizing an oligosaccharide of unknown composition with melibiose also.

Glucose and galactose were the best sources amongst the hexoses and the difference in their structural configuration did not play any important part.

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LEAF SPOT DISEASE OF *ARTOCARPUS HETEROPHYLLUS* CAUSED BY *PHYLLOSTICTA ARTOCARPINA*

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INTRODUCTION

Leaf spot diseases caused by *Phyllosticta* are of wide occurrence and have been reported practically from every country in the world. So far they have not been studied in detail and mostly only the symptoms of such diseases have been recorded.

In India Da Costa and Mundkur (1948) have reviewed numerous species of *Phyllosticta* but they have not studied any of them. Chaudhury (1944) reported *Phyllosticta* sp on the leaves of *Carica papaya*. He found that the disease was more prominent from September to January. Rama Krishnan (1942) reported a leaf-spot disease of *Zingiber officinale* caused by *Phyllosticta zingiberi*. He established that slight control of the disease was possible by spraying with 2 : 2 : 50 Bordeaux mixture. Luthra and Bedi (1932) reported *Phyllosticta rabei* on the leaves of gram. They, however, failed to control the disease. Bose (1949) observed a new leaf spot disease of *Ricinus communis* from Kanpur. It was caused by *Phyllosticta bosensis*. According to him spraying with perenox slightly controlled the disease. Tandon and Bilgrami (1954a) reported *Phyllosticta cycadina* on the leaves of *Cycas revoluta* from Allahabad. They established the pathogenicity of the organism and tried to find out suitable methods for the control of the disease.

Phyllosticta artocarpina was reported by Butler (1916) from India. The specimen was collected by Chibber from Bombay Presidency, but no attempt was made to establish the pathogenicity or to control the disease. The same fungus caused serious losses at Allahabad and a detailed study was undertaken.

MATERIAL AND METHODS

The organism was isolated from the infected leaves of *Artocarpus heterophyllus*. The methods of isolation, purification and subculturing were similar to those of Tandon and Bilgrami (1954b). Artificial inoculations of *Phyllosticta artocarpina* were tried on the different parts of the host. Both young (15-30 days) and old (6-8 months) leaves of *A. heterophyllus* were used. Pathogenicity tests were conducted on injured and uninjured surfaces. Injury was inflicted with the help of a sterilized scalpel. Care was always taken that the injury was not deep. The following different methods of inoculations were tried for inoculating the leaves and stem.

1. Mass inoculation method
2. Spore suspension method
3. Germinating spore suspension method
4. Sprinkling powder of infected leaves
5. Infected leaves tied with healthy ones

The fruits were incoulated by the method described by Granger and Horne (1924), though it was slightly modified on account of the nature of the fruit. A much larger sized cork borer had to be used.

Controls were simultaneously maintained. Reisolations were always made in order to confirm the results.

To study the longevity of spores, some naturally infected leaves of the host showing pycnidia of the fungus were detached from the plants and were stored in the laboratory at room temperature for various periods. Pycnidia from such leaves were picked up after every month and crushed on glass slides. A suspension of the spores was prepared in 0·1% dextrose and the percentage germination at 100% of humidity and 25°C temperature was recorded after 20 hours. The method suggested by Fosberg (1949) was used for evaluating the fungicides in the laboratory.

OBSERVATIONS

Symptoms of the disease :—In most cases, the disease starts as dark brown or chestnut brown spots (0·2-0·5 mm. in diameter). They are scattered on both the upper and lower surfaces of the leaf (vide plate 1, fig. 1) and enlarge to about 1·2 cm. in about 20-35 days. In some cases, the spots coalasce forming irregular patches. Generally, these spots fail to cross the mid rib of the leaf. Black dot like pycnidia appear on the upper surface after about a month of infection. They are slightly raised above the epidermis. With age these spots become grayish, papery and brittle and they begin to die from the centre towards the periphery. Ultimately the infected part falls off and "shot holes" are developed (vide plate 1, fig. 2). This whole process takes 6-7 months. Rarely the infection is marginal or from the tip and in such cases the infected portion falls from the margins or from the tips. Whenever the disease starts from the tip, it spreads on both the sides of the mid rib.

Artificial inoculation :—The organism was inoculated by different method on young as well as old leaves of *Artocarpus heterophyllus*. The results are summarized in Table No. 1.

TABLE No. 1

Showing the percentage of infection of old and young leaves of *Artocarpus heterophyllus* inoculated by different methods.

Type of inoculum	Condition of leaf	Surface of leaf	Percentage of infection	
			old	young
1. Mass inoculation	Injured	Upper	70	50
	Uninjured	Upper	25	15
	Injured	Lower	80	65
	Uninjured	Lower	50	45
2. Spore suspension	Injured	Upper	65	60
	Uninjured	Upper	20	15
	Injured	Lower	65	50
	Uninjured	Lower	50	40
3. Germinating spore suspension.	Injured	Upper	80	65
	Uninjured	Upper	25	20
	Injured	Lower	80	70
	Uninjured	Lower	60	55
4. Sprinkling powder of infected leaves	Injured	Upper	35	30
	Uninjured	Upper	0	0
	Injured	Lower	40	30
	Uninjured	Lower	25	20
5. Infected leaf tied with healthy	Injured	Upper	30	25
	Uninjured	Upper	0	0
	Injured	Lower	35	25
	Uninjured	Lower	20	15

The table shows that percentage of infection was greater, whenever the inoculations were made over injured surfaces. The table also indicates that all the five methods of inoculation were not equally effective for the infection. Percentage of infection was greater, when inoculations were made by methods 1, 2 or 3 in such cases the symptoms of the disease appeared 3-4 days earlier.

It is also evident from the above table that the older leaves were more severly infected than the younger ones.

Reisolations from the infected regions invariably gave *Phyllosticta artocarpina.*

Besides leaves the organism was inoculated on stems (both old and young) as well as on fruits of *Artocarpus heterophyllus* (both ripe and unripe) but it could not infect any of those parts.

Cross inoculations :—*Phyllosticta artocarpina* was cross inoculated on the leaves of *Cycas revoluta, Mangifera indica, Ficus religiosa, F. bengalanensis, Psidium guajava, Butea frondosa* and *Citrus* sp, but it could not infect any of them.

During artifical inoculation it was also observed that infection did not take place if either moist cotton pads were not placed or water was not regularly sprayed on the inoculum for the first four days.

Experiments on the longevity of the spores showed that the percentage of spore germination decreased with the storage time but the spores could germinate even after 7-8 months.

Source of infection :—Under natural conditions, only the leaves of *A. heterophyllus* were found to be infected and there was no damage to any other part of the plant. It was also observed that some of the infected leaves of the host, remained attached to the plant throughout the year, though in most cases the infected portion of the leaf got detached from the healthy part, but even in such cases, the junction of infected and healthy portion, contained a layer of infected region, which contained enough viable spores. Experiments on the longevity of the spores showed that under suitable temperature and humidity, the spores retained their viability and could germinate even after 7- 8 months. It was also observed that infection could occur readily, if the spores from attached infected parts or from stored stock (upto 7 months old) were transferred to healthy plants during rainy season. It is, therefore, concluded that under natural conditions, the spores left in the soil or on the attached diseased parts of the leaves are responsible for the carry over of the disease from season to season. The maximum infection under natural conditions starts during rainy season.

Control measures :—Laboratory evaluation of certain fungecides indicated that, 3 : 3 : 50 Bordeaux mixture, 0·2% tillex, 0·2% copper sandoz and 0·33% perenox, prevented the growth of the organism. They were, therefore, used against the leaf spot disease caused by *P. artocarpina*. The results have been summarized in table No. 2.

TABLE No. 2

Showing the effect of spraying various fungicides on the leaves of *Artocarpus heterophyllus* inoculated with *P. artocarpina.*

Sign (+) or (-) have been used to denote the appearance or absence of disease respectively.

Time of incoulation	Fungicides sprayed			
	bordeaux mixture 3 : 3 : 50	Peronox ·33%	Tillex ·2%	copper sandoz ·2%
1. Just after spraying	-	-	-	-
2. 24 hrs. after spraying	-	-	-	+
3. 48 hrs. after spraying	-	-	+	+
4. 96 hrs. after spraying	+	+	+	+
5. 1 week after spraying	+	+	+	+
6. Just before spraying	-	-	-	-
7. 24 hrs. before spraying	-	-	-	-
8. 48 hrs. before spraying	-	-	+	+
9. 96 hrs. before spraying	-	+	+	+
10. 1 week before spraying	+	+	+	+

It is evident from Table No. 2 that bordeaux mixture (3 : 3 : 50) and 0·33% perenox served as effective fungicides, provided they were sprayed 2-3 days before or after artificial inoculations. Any treatment even a week before the inoculation was unable to check the disease. Under natural conditions it is difficult to know the exact time of infection, and hence a number of applications will be necessary during the most susceptible period.

DISCUSSION

Present studies established that *P. artocarpina* was pathogenic to the leaves of *Artocarpus heterophyllus*. It was incapable of infecting other plants. Chromatographic analysis of the host revealed that concentration of sugars was greater during June-July than during January-February, (Bilgrami 1956). It has also been observed that it grows better on higher concentrations of sugars. It appears that initiation of infection during rainy season may be connected not only with high humidity and suitable temperature, but may also be due to presence of tender tissues as well as high concentration of sugars in the host, during that time of the year. The results also indicated that *P. artocarpina* was exclusively a leaf parasite because, it failed to grow on the other parts of the host and in this respect the results were similar to those of Rama Krishnan (1942), Luthra and Bedi (1932), Bose (1949) and Chaudhury (1944), who worked with *Phyllosticta Zingiberi*, *P. rabei*, *P. bosensis* and *Phyllosticta* sp respectively.

It has also been established that the organism was capable of surviving in the soil or on the host for about 8 months and infection was possible through the contact of healthy and diseased tissues. In nature many diseased leaves have been found to remain attached to the host and they appear to be responsible for the subsequent infection of the new areas. Mere spraying the plants with bordeaux mixture or perenox can not, therefore, give successful results, unless suitable hygenic conditions are provided for the plants.

SUMMARY

Phyllosticta artocarpina was isolated from the infected leaves of *Artocarpus heterophyllus* at Allahabad and other neighbouring places. Pathogenicity of the organism was established on the leaves of the host. Cross inoculations on a number of other plants were unsuccessful. Spores on the diseased leaves were viable for 7-8 months and they served as the source of inoculum for fresh infections. Field trials were made on the basis of laboratory evaluation of fungicides and control measures have been suggested. Spraying with 0·33% perenox or 3 : 3 : 50 bordeaux mixture could greatly prevent the infection.

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